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Department of Biological Sciences, Wolson Ecology Laboratory
University of Exeter, Devon, U.K.

Assimilation of ^{14}C-labelled leaf fibre by the millipede *Glomeris marginata* (Diplopoda, Glomeridae)

J. M. Anderson and D. E. Bignell

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1. Introduction

Deciduous tree litter may be considered a low quality food resource for saprotrophic animals since dead leaves have a lower content of low molecular weight oligosaccharides and available nitrogen than living leaves, and are correspondingly richer in lignocellulose. For most litter-feeding arthropods other than termites, assimilation efficiencies in the range 10—30% are commonly quoted (reviews by Edwards 1974, Harding & Stuttard 1974), but considerable variations can occur both within and between different taxonomic groups. Thus while van der Drift (1950), Gere (1956), Bocock (1963) and McBrayer (1973) found that the dry weight assimilation of litter by millipedes was less than 10%, considerably higher values of between 30 and 83% are given by Striganova (1971) and Striganova & Valiachmedov (1976). Various studies of woodlice have revealed a similar diversity of values, with reports of assimilation efficiencies from 10—70% (Edwards 1974).

A major problem in the interpretation of these results is that the studies have employed litter defined by plant species rather than biochemical composition, yet the latter will change during the course of decay and may differ between parent soil types. Since leaf litter is rarely attacked by temperate soil animals until weeks or months after falling, some preliminary decomposition must apparently take place before the material is palatable. The delay is often attributed to the presence of polyphenolic feeding inhibitors which must first be removed by leaching or microbial metabolism (Edwards et al. 1970, Satchell 1974), but is more likely to result from the rapid accumulation of microbioal biomass and by-products of microbial catabolism as the plant structural compounds are de-polymerized (Anderson 1973, Neuhauser & Hartenstein 1978, Swift et al. 1979). The onset of decay on the forest floor is accompanied by rapid fungal colonization, such that a major proportion of the leaf nitrogen and phosphorus is immobilised in mycelium within a few weeks and this tissue therefore becomes a significant and increasing resource potential for the fauna (Swift et al. 1979). Thus the net assimilation of litter-feeding animals depends on the digestibility of a wide range of materials many of which may be of secondary (fungal) origin and determinations which are based on dry weight comparisons of food and faeces or on calorimetry (van der Drift 1950, Gere 1956, Shaw 1970) contribute rather little to an understanding of the nutritional biology of soil animals.

A gravimetric determination of assimilation efficiency in *Glomeris marginata* (Villers) was employed by Bocock (1963) but combined with chemical analyses of food leaves and faeces. Although the net effects of gut passage were small in terms of weight changes (in common with the results of Dunger 1958) the assimilation efficiency of the holocellulose fraction was found to be 28.4%. Striganova (1971) and Striganova & Valiachmedov (1976) give much higher values for other millipedes, using similar methods, with as much as 80% of ingested cellulose being degraded in the gut. Unfortunately there are considerable errors which may be associated with proximate analysis of dead plant materials: the de-

gradation products of cell wall materials are frequently unknown and the materials themselves may be biochemically modified or partially broken down during the extraction process (Bocock 1963).

The use of ^{14}C-labelled plant materials to investigate assimilation in decomposer animals has been reported by Engelman (1961), Calow & Fletcher (1972) and Reyes & Tiedje (1973, 1976). Although the method offers increased sensitivity and obviates the need for fraction, in these experiments the label was not restricted to a single fraction of the food material; hence the increased accuracy of the determination of assimilation was offset by a lack of substrate specificity.

In this paper we report the determination of the assimilation of ^{14}C-leaf fibre by *Glomeris marginata*. To confine the label to a relatively well-defined fraction of non-microbial origin, the fibre was prepared by systemic labelling of living detached leaves and applied tolpically to standard unlabelled leaf litter discs for consumption by the animals. The results show that about 36 % of the labelled substrate was utilized during a single transit of the alimentary canal.

2. Material and methods

2.1. Animals

Glomeris marginata (Villers) were collected during wet weather in mid-August and established as stock cultures in large glass tanks containing moist, well rotted oak and beech litter at ambient, outdoor temperatures. Experimental animals were separated from stocks 24 hours prior to use and kept in smaller containers without leaf litter.

2.2. Labelled leaf fibre

^{14}C-leaf fibre was prepared at the Radiochemical Centre, Amersham, U.K., by allowing detached leaves of *Canna indica* to photosynthesize for hours in an atmosphere containing $^{14}CO_2$. Soluble carbohydrates were removed by extraction with hot aqueous ethanol to leave a residue of leaf fragments which were dried and shipped at a specific activity of approximately 0.35 mCi mg^{-1}. In the laboratory the leaf fragments were pulverized and mixed intimately with unlabelled polysaccharide in a Toothmaster High Speed Dental Amalgamator and the mixture diluted with additional unlabelled polysaccharide to a final specific activity of about 2 mCi g^{-1}. Unlabelled polysaccharide consisted of a 50/50 (w/w) mixture of ball-milled ashless filter paper and hemicellulose (Bignell 1977). Homogeneity in the final labelled mixture was achieved by turning on an electric rolling mill for 48 hours and checked by counting replicate aliquots in gelled liquid scintillation fluid (Bignell 1977). Further aliquots (about 100 mg) were extracted exhaustively in hot 80 % ethanol and hot distilled water (successively) until no significant radioactivity remained in the supernatant. The residue was then suspended in 2 ml cold distilled water, a portion of the suspension drawn into a Pasteur pipette and 1 drop (36 μl) applied to the surface of each of a series of 1 cm diameter leaf discs cut from oak litter with a cork borer and placed in corresponding 1 cm wells bored in a solidified slab of 1.5 % water agar. Using a sterile wire loop, the applied drop of polysaccharide suspension was spread evenly over the leaf disc and the disc left within the wells for a few minutes during which excess fluid was absorbed by the agar, depositing a thin layer of moist polysaccharide over the surface of the disc.

2.3. Consumption of fibre and analysis of radioactivity

Five large *Glomeris* were placed in a flat 10 cm square plastic box containing a moist plaster of Paris substratum. Five labelled leaf litter discs were added to the culture, which was then incubated in the dark for 24 hours at 22 °C. Preliminary experiments showed that a 1/1 ratio of animals to discs was optimum for ensuring that the amount of unconsumed food remaining at the end of the incubation period was a minimum and that most of the ingested litter had passed through the gut and been voided as faecal pellets. Following the incubation, faeces were collected from the culture box with fine forceps, added to 2 ml distilled water in a McCartney bottle containing a plastic bead and homogenized by 5 minutes of vigorous mechanical shaking. For counting, 0.1 ml aliquots of the resulting suspension were added to 10 ml Bray's scintillation solution and this mixture gelled by the addition of excess Cabosil gelatinizing powder, accompanied by vigorous shaking. To collect haemolymph and minimise the loss of this fluid during dissection, the animals were first punctured the ventrolateral cuticle and fluid drawn off in a capillary pipette. Samples of haemolymph obtained in this manner from each animal were pooled and added to 0.2 ml Soluene in a scintillation vial. When the haemolymph was completely dissolved, 10 ml of toluene-based scintillation fluid were added for counting. The entire alimentary canal was removed from each animal by dissection,

added to a pool in 2 ml distilled water, homogenized and prepared for counting as described for faeces above. The remaining carcasses were also pooled and extracted overnight in 2 ml Soluene at room temperature. 0.1 ml aliquots of this solution were added to 10 ml of toluene-based scintillation fluid.

2.4. Treatment of evolved $^{14}CO_2$

Preliminary experiments to determine the evolution of $^{14}CO_2$ following the consumption of ^{14}C-fibre were carried out in standard Warburg flasks containing 0.3 ml 2 N KOH in the sidearm. Five *Glomeris* and five labelled leaf litter discs were placed in a flask which was then sealed with a rubber stopper and incubated in the dark for 24 hours at 22 °C. Following the incubation 0.1 ml aliquots of the alkali were applied to 1 cm diameter glass fibre discs and counted in Bray's solution. Control flasks were prepared containing labelled leaf litter discs and alkali only. It was found that millipedes confined within the Warburg flasks became contaminated with faecal material such that it was impossible to distinguish reliably between radioactivity incorporated into tissues and that voided in the excrement. Counts recovered as $^{14}CO_2$ were therefore expressed as a proportion of the total counts recovered from haemolymph, tissues, gut and faeces, corrected for background (i.e. microbial) respiration by subtraction of the counts recovered as $^{14}CO_2$ in control flasks. For a 24 hour incubation period this proportion was found to be 10.7 $\pm$ 3.1 % (mean of five determinations), and was applied as a correction in subsequent experiments (2.3 above) in which the evolution of $^{14}CO_2$ was not determined directly.

3. Results

Assimilation efficiency was calculated as:

$$\frac{\text{Counts in haemolymph} + \text{counts in tissues} + \text{counts in } CO_2 \text{ (estimated)}}{\text{Total counts recovered (including } CO_2)}$$

The formula therefore assumes that the total counts recovered from CO_2, animals and faeces are equal to the total counts ingested, avoiding the difficulty of determining consumption as the difference of radioactivity between subsampled whole food and the unconsumed material remaining after incubation. However it does not take account of radioactivity that may be leached from faeces into the substratum before analysis or of increased respiration in faecal pellets that may result from comminution of leaf material or stimulation of microbial growth during intestinal transit (Hanlon & Anderson 1980, Anderson & Bignell 1980). These factors would tend to increase the number of counts actually ingested in comparison with those recovered for analysis and thus lead to an overestimate of assimilation, but these errors are likely to be offset by the loss from the numerator of counts absorbed into the gut wall but not transported into the haemolymph or other body tissues at the time of analysis.

Table 1. Distribution of radioactivity following ingestion of ^{14}C-fibre with leaf litter discs by *Glomeris marginata*

Total counts ingested	Counts in tissues and haemolymph	Counts in gut and faeces	Counts in CO_2 (estimated)	Assimilation efficiency (%)
105,829 $\pm$ 21,167	27,729 $\pm$ 4,745	68,454 $\pm$ 15,535	10,229 $\pm$ 2,046	35.8 $\pm$ 3.4

Note: The data represents a single pulse transit of the gut. Mean $\pm$ 1 SD of radioactivity corrected for quenching and background (d.p.m's). n = 10 (determinations), 5 animals per determination.

Table 1 shows that the mean assimilation efficiency of ^{14}C-fibre was determined as 35.8 % for a single, pulse transit of the alimentary canal. This agrees broadly with the findings of Bocock (1963) that 28.4 % of ingested holocellulose was assimilated by the same species feeding *ad libitum* on ash litter. The relatively low proportion of ingested ^{14}C-fibre respired by the animal during the incubation period may be cited as justification for determining $^{14}CO_2$ in a separate experiment, since the errors thus introduced will have only a minor effect on the cumulative error of the determination of assimilation. The figure agrees closely with data given by Reyes & Tiedje (1976) for $^{14}CO_2$ evolution by the woodlouse *Tracheoniscus rathkei* on the first day following consumption of ^{14}C-labelled cottonwood leaves, although it might be expected that smaller arthropods would respire a greater proportion of assim-

ilated energy in the same period of time (BERTHET 1971). The precise partitioning of available substrates between respiration, production and storage will not affect the calculation of assimilation efficiency provided that all of these are determined with comparable accuracy.

4. Discussion

The results show that degradation of the non-labile residues of plant material by *Glomeris marginata* is significant but modest, in keeping with the view that there is relatively little difference in chemical composition between the food and faeces of this millipede. In *Tracheoniscus rathkei* REYES & TIEDJE (1976) showed that fractions of plant material containing hemicellulose were more rapidly metabolized than those in which lignocellulose was present and that intestinal micro-organisms, in so far as could be assessed by the administration of antibiotics, competed with the host for the more readily digestible substrates rather than assisting symbiotically in the breakdown of refractory substances. A similar phenomenon may occur in *Glomeris marginata* and the woodlouse *Oniscus asellus* where bacterial counts in the gut increase to about 10 times those of the food litter and further amplification, to about 100 times control levels, takes place in fresh faeces (ANDERSON & BIGNELL 1980; ANDERSON & INESON, in press). Thus considerable carbon utilization by bacteria must occur during gut transit but the origin of the resources, whether from the animal or the food material, remains to be determined.

Criticism may be levelled at the use of detached living leaves for the labelling of plant fibre on the grounds that little cell wall synthesis would be occurring in the constituent tissues and that the label is therefore unlikely to be distributed evenly along the polymeric chains or microfibrils (REYES & TIEDJE 1976, BIGNELL 1977). The estimate of assimilation of fibre provided by uptake of label into the animal may therefore more accurately reflect the activities of exoglucanase or exoxylanase occurring unilaterally in the gut lumen than the complex of synergistic enzymes normally associated with the complete degradation of cell wall components. An advantage of ^{14}C-leaf fibre is that it resembles natural structural polysaccharides in configuration more closely than fractionated hemicellulose, cellulose or lignin. The topical application of labelled polysaccharide mixture to unlabelled leaf disc may present an additional difficulty in that access to the labelled substrate by digestive enzymes may be easier than in wholly natural food. However the usefulness of the technique is that it permits the even application of a relatively specific labelled fraction in a way that leaves the disc palatable to the animal (see below) and equalizes the inbalance of label between leaf veins and non-vascular portions of the lamina that would be expected in any systematically-labelled material.

The nutritional role of coprophagy and the extent to which ingested micro-organisms (especially fungi) are digested both remain unclear. Preliminary experiments using ^{3}H-labelled bacteria presented on leaf disc indicated that approximately 53 %, 53 % and 29 % respectively of *Pseudomonas syringae*, *Erwinia herbicola* and *Escherichia coli* were assimilated by *Glomeris* (ANDERSON & BIGNELL, unpublished data). MCBRAYER (1973) showed that the assimilation efficiency of the millipede *Apheloria montana* could be increased some 3 % when both faeces and litter were available for ingestion, compared with litter alone: however the nature of the fractions utilised was not established. The difference could reflect the digestion of bacterial biomass rather than an enhancement of microbially mediated catabolism of structural polysaccharides in the litter.

Circumstantial evidence suggests that the extent of microbial colonization influences the palatability of food since well rotted leaves are preferred to freshly fallen litter and washed or mechanically fragmented material, although previously acceptable, are not consumed to any significant extent by *Glomeris* until composting has occurred (VAN DER DRIFT 1950, ANDERSON & BIGNELL unpublished data). An understanding of the nutritional strategy of *Glomeris marginata* or any other macroarthropod detritivore is therefore incomplete without information on the digestion of microbial tissues ingested with leaf material and

the possible roles of acquired microbial enzymes in intestinal processes (HASSAL & JENNINGS 1975, MARTIN 1979). Further work is in progress to address these questions.

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Addresses of the authors: J. M. ANDERSON, Department of Biological Sciences, Wolfson Ecology Laboratory, University of Exeter, Devon, U.K. EX4 4PS. D. E. BIGNELL, Department of Zoology, Westfield College, University of London, Hampstead, London, U.K. NW3 7ST.

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^{14}C-labelled fibre was prepared from living leaf tissue and applied topically to standard discs of well-rotted oak litter. When the discs were consumed by millipedes under laboratory culture conditions, a mean of 38% of the ingested label was assimilated into the haemolymph and body tissues, or respired by the animals. It is argued that the method does not allow the incorporation of label into microbial tissue prior to ingestion and therefore accurately assesses the ability of the animal to assimilate the primary structural components of leaf litter. The nutritional significance of these materials and of microbial biomass ingested with the litter is discussed.

Key words: **Diplopoda, Glomeris marginata, assimilation, consumption, ^{14}C-labelled leaf fibre, deciduous litter, palatability, microbial mediated catabolism, gut transit.**